SCREENING TANNIN-UTILIZING FILAMENTOUS FUNGI FOR PROTEIN PRODUCTION FROM AQUEOUS CAROB EXTRACT

by S. MARAKIS*

SUMMARY. — The carob bean water soluble sugar and tannin extraction procedure by autoclave proved productive and beneficial. For the initial screening 930 isolates of filamentous fungi from various regions of Crete were cultured on aqueous carob extract. Values of growth parameters like : specific growth rate (μ), biomass yield (y), protein yield (yp) and amount of carob tannin utilization were higher in cultures of carob bean and storehouse soil sample isolates than those from carob tree plantation soil samples. The microorganism eventually selected was *Aspergillus carbonarius* (AsDT10) which gave high biomass and protein yield (y = 69.5 % and yp = 25.6 %), specific growth rate ($\mu^{max} = 0.300 h^{-1}$) and tannin utilization (95 % on initial concentration) values. The mycelium was rich in true protein (37.5 %) with balanced amino acid profile but poor in nucleic acids (5.1 %) and ash (4.8 %). The nutritional value of the *A. carbonarius* biomass determined in feeding trials with rats gave : protein efficiency ratio (PER = 1.95 ± 0.1), net protein utilization (NPU = 0.63 ± 0.02), biological value (BV = 0.70 ± 0.01) and true digestibility (TD = 0.85 ± 0.02).

RÉSUMÉ. — L'extraction à l'autoclave des sucres et des tanins solubles contenus dans l'eau des caroubes, s'avère économique et productive. Neuf cent trente champignons filamenteux ont été isolés de différentes régions de la Crète, et ils ont ensuite été cultivés dans un extrait aqueux de caroubes, pour le «screening» initial. Les valeurs de paramètres de croissance des isolements effectués à partir de caroubes et de sol de caroubes stockées, telles que : le taux de croissance spécifique (μ), le rendement en biomasse et en protéine (y, yp respectivement), et le pourcentage des tanins utilisés, sont supérieures à celles des isolements effectués à partir de sol sous les caroubiers. Finalement, on a sélectionné le microorganisme *Aspergillus carbonarius* (AsDT10), qui a donné des rendements en biomasse et protéine considérables (y = 69.5 % et 25.6 % respectivement), un taux de croissance spécifique ($\mu^{max} = 0.300 h^{-1}$), et une utilisation de 95 % des tanins du milieu. Le mycélium de *A. carbonarius* est riche en protéine réelle (37.5 %) avec une teneur équilibrée en acides aminés, mais pauvre en acides nucléiques (5.1 %) et en cendres (4.8 %). L'estimation nutritionnelle de la biomasse de *A. carbonarius* a été déterminée par des essais sur rats; les valeurs des indices de nutrition sont : le coefficient d'efficacité protéique (PER = 1.95 ± 0.1), l'utilisation protéique nette (NPU = 0.63 ± 0.02), la valeur biologique (BV = 0.70 ± 0.01) et le taux réel de digestibilité (TD = 0.85 ± 0.02).

KEY WORDS : Carob, carob bean, tannins, fungal protein.

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INTRODUCTION

The need for more protein is universally recognised. Many schemes have been proposed for supplementing the world's increasing demand for protein with the microbial protein production. Many microorganisms have been cultured for this purpose on a wide variety of inexpensive substrates mainly of agricultural origin.

An agricultural product of low commercial value in Greece is the carob bean (fruit of *Ceratonia siliqua* L. tree). The carob tree naturally grows on barren soils of the Mediterranean regions as well as in other parts with a similar climate. Greece is the fourth largest carob bean producing country in the world. The ripe carob pod (pericarp), although rich in water soluble sugars (more than 50 %; mainly sucrose 70 %; glucose 10 % and fructose 10 % on the total sugars) has a very low crude protein content (about 6 %) on dry weight (CHARALAMBOUS & PAPACONSTANTINOU, 1966; KALAITZAKIS, 1979). The pericarp also contains high levels of total tannins (up to 6 %) mainly condensed (TAMIR & ALUMOT, 1970; TAMIR & al., 1971) which minimizes the nutritional value of carobs because of protein coagulation and astringent taste on ruminants and pigs (OSLAGE & BECKER, 1958; VOHRA & al., 1966; TAGARI & al., 1965; BORNSTEIN & al., 1965; TAMIR & ALUMOT, 1970). In the past aqueous carob extract have been used for studies related to fungal protein production (SEKERI-PATARYAS & al., 1973; DROULISCOS & al., 1976; MACRIS & KOKKE, 1977, 1978) but no studies concerning carob tannins utilization have been published.

Aim of this investigation is the improvement of this agricultural product by studying the growth of filamentous fungi isolated from natural material. These microorganisms must be able to grow in aqueous carob extract and utilize carob tannins; also, to produce tannin-free fungal biomass rich in protein with a balanced amino acid profile.

This paper describes the screening of filamentous fungi. In addition the isolation of the most effective fungus in a) tannin utilization and protein rich biomass production, b) the kinetics of growth and biomass chemical composition was of the main targests of this investigation. Evaluation of the nutritional quality of the produced biomass was also carried out using rats.

MATERIALS AND METHODS

— Microorganisms :

Three hundred samples of natural material i. e. carob beans, soil collected from carob bean storehouses and underneath carob trees, were used for the isolation of 930 filamentous fungi which have been classified in 35 species (CHARPENTIÉ & MARAKIS, 1980). The samples of the natural material were collected from various areas of Crete.

– Preparation of aqueous carob extract :

The carob beans were smashed in a manufacture mil. A 4-Kg quantity of chopped and deseeded carob pods was mixed with 16 liters of deionized water and autoclaved (P = 1 Atm, T = 121°C, t = 30 min). The slurry was passed through cheesecloth and the spent carob resuspended in 10 liters of deionized water and autoclaved once again. The two filtrates were mixed and a syrup containing 10-11 % total sugar and 0.9-1 % total tannins (both hydrolysed and condensed) was obtained.

– Growth media :

The media A, B, C were used. These media were prepared by mixing the aqueous carob extract and some salts. The carob extract was diluted with salt-water solution to a concentration of total sugars 1, 3, 4, 5, 6, 7 and 9 % w/v, so as the composition (g/l) of the media in sugars and salts becomes :

COMPONENTS			MEDIA A	MEDIA B*	MEDIA C
Carob sugars			30	10-90	50
Added salts and urea	Nitrogen sources	$(NH_4)_2SO_4$	5	–	–
		$NaNO_3$	–	2.43-21.86	–
		Urea	–	0.86- 7.72	–
		$NH_4H_2PO_4$	–	3.30-29.60	18
		NaH_2PO_4	1	0.41- 3.70	–
		$MgSO_4.7H_2O$	–	0.16- 1.44	0.80
		NaCl	–	–	0.75

* In media B : concentration of added nitrogen sources was calculated on the base of ratio C:N = 10:1, where C = carob sugar carbon. Concentration of both NaH_2PO_4 and $MgSO_4.7H_2O$ were calculation from the ratio Sugar/Salt = 1/0.041 and 1/0.016 respectively.

The carob extract was sterilized by filtration through membrane filter (0.2 μ pore size Gelman Michigan) while the salt solution by autoclave and then these were mixed. The total tannin concentration of each medium constituted about 1/10 of that of the total sugars. The pH was buffered to 5-5.2.

Medium A was used for the primary mass-screening. The selected isolates were cultured in medium B on purpose to optimize culture conditions (inoculum size, incubation time, initial concentration of total sugars and nitrogen and salt sources). The microorganism eventually selected was finally cultured in medium C.

– Preparation of inoculum :

Peripheral growth zone spores from Czapek-Dox agar cultures were obtained after 5-8 days of incubation at optimum temperature for each micro-

organism. Spores were suspended in quater-strength Ringer's solution for 1 min in a Waring blender.

– Batch cultivation :

During primary screening and experiments for the optimization of the culture conditions microorganisms were grown in 100 ml Erlenmeyer flasks containing 20 ml of medium. These flasks were inoculated with $0{,}5.10^6$ -$0{,}2.10^7$ spores/ml of medium and incubated on reciprocal shaker (120 strokes per min.). Temperature and incubation time were regulated in relation to experiment. Each of the above experiments was run in triplicate (five flasks per run). The results were represented as mean values ± stardard error. Cultivation of the microorganism eventually selected for further study (kinetics of growth and chemical composition) was carried out in a fermentor (Tate and Lyle Co, England) consisting of 7 lit-fermentation vessels equipped with agitation and aeration devices and automatic temperature control. The medium (C) was inoculated with spores ($0{,}2.10^7$ spores/ml of medium). Agitation was effected with two impellers at 500 rpm for the first 15 h after inoculation and 800 rpm thereafter. The culture was aerated with compressed air sterilized by filtration (Microflow Ltd. Cat. No L 32, England). The aeration rate was kept at a level of 0.5 l air per 1 l of medium per min. Foaming during microorganism cultivation was controlled manually by adding antifoaming agent (Silicon-Entschäumer Merck). During cultivation pH was automatically controlled at 4.8-5.0 by the addition of NaOH 1N buffer solution. The temperature was stabilized at 32 ± 0.5°C. Samples (50-100 ml) were aseptically withdrawn from the fermentation vessel at three hour intervals during the first 24-hour period. Six and 12 hour intervals withdrawals were carried out at the next 24-hour (25-48) and 36-hour (49-84) period respectively.

– Harvesting and drying of biomass :

The mycelial mats were harvested by suction filtration through Whatman no. 1 filter paper, washed with equal to the culture volume of distilled water and dried by lyophilization to constant weight.

– Analytical methods :

- Total sugars in carob and culture filtrates were determined as described by DUBOIS & al. (1956) method after tannin removal according to the *Association of Official Agricultural Chemists* (AOAC, 1970) method.
- Total nitrogen was estimated by the method of VARLEY (1966).
- True protein was determined by the method of LOWRY & al. (1951) as modified by GORSUCH & NORTON (1969).
- Cold trichloracetic acid (TCA) soluble and alcohol insoluble fraction of mycelial nitrogen was determined according to the method of DELANEY & al. (1975).
- Nucleic acids were extracted by the method of DELANEY & al. (1975).

RNA was estimated by the method of GOTTLIEB & VAN ETTEN (1964), and DNA by the diphenylamine method (DISCHE, 1955) using bakers's yeast RNA and calf thymus DNA (both Sigma Chemical Co. Ltd. St. Louis U.S.A.) as standards.

- Purines were determined by the method of TREVELYAN (1975).
- Moisture was estimated by oven drying at 105°C to constant weight.
- Ash was determined by ignition at 550°C in an electric muffle furnace.
- Non-protein nitrogen (NPN) was estimated by the *AOAC* (1970) method.
- Total lipids were extracted by the method of WINTER (1963) while the saponification of lipids and esterification of fatty acids in methyl esters carried out by the methods of STOFFEL & al. (1959) and BAYER (1962). Quantitative and qualitative determination of methyl esters was made by gas-chromatography (KAISER, 1965; KULL & JEREMIAS, 1972). Fatty acid content was expressed as the percentage of the total fatty acids.
- Amino acid analysis of the dry mycelium was carried out on hydrolysed samples using a Technicon automatic analyser. The sample proteins were hydrolysed in N_2 saturated environment with 6N HCl at 105°C for 24 h. The hydrolysate was filtered and the HCl-acid removed by evaporation under reduced presure at 40°C. The residue was taken up in 4 ml 0.01 N HCl (pH = 1.9). The high loss of sulfur-containing amino acids which occurs during acid hydrolysis of proteins was prevented by performic acid treatment before hydrolysis (LEWIS, 1966; SCHRAM & al., 1954). Tryptophan determination was made according to SPIES (1967) method. Fluoro-dinitro benzene available lysine was estimated by the method of CARPENTER (1960) as modified by BOOTH (1971). The essential amino acid index (EAAI) was calculated by the method of OSER (1951).
- For determination of caloric content 1 g freeze-dried biomass was burned in a Parr oxygen bomb calorimeter at 32 atm oxygen pressure, standardized with benzoic acid tablets. (239 Kcal = 1 MJ of metabolizable energy (MALEFAKI-PERELA, 1981)).
- B-group vitamins were determined by BELL (1974) method.
- Water-soluble total tannins were extracted by refluxing 1 g freeze-dried biomass in 500 ml of distilled water for 1 h. The total tannins in mycelium extract, carob extract and culture filtrates were estimated by the Folin-Denis colorimetric method (*AOAC*, 1970), using tannic acid as standard. The flavonols were estimated according to SWAIN & HILLIS (1959) method using catechin as standard.
- Carob cellulose and lignin were determined by the JERMYN & ISHERWOOD (1956), VAN SOEST (1963) and UPDEGRAFF (1969) methods.

— Nutritional evaluation :

Male and female rats of the Hooded strain weighing 45-50 g for protein efficiency ratio (PER) and net protein utilization (NPU) tests and 80 ± 5 g for the N balance study were used. The further procedure followed (rat feeding

and calculation of nutritional indices) was as described by MALEFAKI-PERELA (1981), DROULISCOS & MALEFAKI (1980), EGGUM (1973), MILLER & BENDER (1955), BENDER & MILLER (1953).

The experimental diets used in these trials were : stock protein-free, soya bean oil meal and *Aspergillus carbonarius* (AsDT10) biomass. Stock protein-free composition was (g/kg) : maize starch, 660; sucrose, 200; cellulose, 50; maize oil, 50; mineral salts, 30; vitamin supplement, 10 (DROULISCOS & MALEFAKI, 1980). Soya bean oil meal and *A. carbonarius* biomass diets were prepared by adding soya bean oil meal (189 g) and *A. carbonarius* biomass (196 g) in stock protein-free diet to make up 1 kg diet. The total nitrogen content (by analysis) of the diets was about 14.3 g/kg of diet except of the stock protein-free diet (0.78 g/kg). Egg powder (40 g/kg) was included in the stock protein-free diet for the determination of the metabolic and endogenous N.

RESULTS AND DISCUSSION

Extraction of water-soluble carob sugars and tannins :

The aqueous carob extract which was obtained by the method described in material and methods contained 10-11 % total sugars and 0.9-1 % total tannins. On the base of these data, carob pods contained about 58.5 % and 5.8 % on dry weight water extractable sugars and total tannins respectively. SEKERIPATARYAS & al. (1973) and DROULISCOS & al. (1976) used different methods for carob sugars extraction. These investigators supplied data about the concentration of sugars in the carob extract. On the base of DROULISCOS & al. (1976) data about 31 % and 0.6 % of total sugars and tannins respectively must have been extracted while the concentration in deseeded carob pods has been reported to be 55 % and 6 % respectively (CHARALAMBOUS & PAPACONSTANTINOU, 1966; TAMIR & ALUMOT, 1970). The extraction procedure used in the present investigation resulted in more than 98 % of the carob pod water soluble sugars and tannins removal. Spent carob contained mainly cellulose and lignin. This residue constituted 25 % of the initial carob pod weight which was extracted.

Selection of microorganism :

The procedure used for the selection of suitable organism was :

a) An initial screening based on the 930 isolates

b) An optimization of culture conditions for the best isolates (strains). These cultivations did not present any problems.

Initial screening : The 930 isolates were cultivated in submerged shaking batch cultures in medium A at the optimum temperature for each isolate for 36 h. The strain *A. niger* (M1) simultaneously was cultivated under the same cultural conditions. This microorganism was used as a reference microorganism

for it has been previously studied in the Institute of General Botany, University of Athens for «yields of fungal protein from carob sugars» (SEKERI-PATARYAS & al., 1973).

The criteria accepted for the selection of the best strains were :

- The mycelium dry weight
- The biomass yield (mycelium conversion efficiency) :

$$y = \frac{\text{(g) mycelium dry weight}}{\text{(g) consummed sugars}} \times 100$$

- The protein yield (protein conversion efficiency) :

$$yp = (y).\ (\text{percentage mycelium crude protein content})$$

- Percentage of total tannin reduction of the medium.

Among the isolates only those with mycelium dry weight, total substrate tannin reduction, biomass yield (y) and protein yield (yp) more than 50 % of those determined for *A. niger* (M1) were selected. Finally only 60 strains remained which have been classified to belong in 35 species as determined by CHARPENTIÉ & MARAKIS (1980).

Mycelium dry weight	: 3.7-14 mg/ml of medium
Crude protein (Nx6.25)	: 25 -47.5% (on dry biomass)
Biomass yield (y)	: 31 -60%
Protein yield (yp)	: 10 -32.5%
Percentage of tannin reduction	: 19 -70.2% (on initial concentration of medium)

Table I — Growth parameter mean variation of 60 isolates of filamentous fungi cultured in medium A for 36 h.

Tableau I. — Variation moyenne de paramètres de croissance dans 60 isolements de champignons filamenteux cultivés pendant 36 h, dans le milieu A.

The results presented in Table I reveal that mycelium dry weight (14 mg/ml of medium), biomass yield (y = 60 %) and percentage of crude protein content (47.5 %) were higher than those observed in filamentous fungi cultures on various natural substrates (SEKERI-PATARYAS & al., 1973; SHUKLA & DUTTA, 1967; CRUZ & al., 1967; HANG & al., 1975). In addition : a) In 50 % of the strains the mycelium dry weight ranged between 6-10 mg/ml while in 17 % was higher than 11 mg/ml of medium. b) The percentage of protein content was higher in *Fusarium* strains, but the total mycelial protein (mg/ml of medium) was higher in *Aspergillus* strains due to higher mycelium dry weight production.

c) One third of the strains revealed biomass yield (y) to be 45-50 % which is in agreement with the ones referred microbial cultures on synthetic media (SOLOMONS, 1975) while in 27 % ranged 50-60 %. d) Ten isolates obtained from carob tree-plantation soil failed to grow on carob extract (medium A) although they were successfully grown on synthetic Czapek-Dox type medium. Tannin reduction in medium A was significantly higher in cultures where isolates from decaying carobs and carob bean storehouse soil were used. The same was not true for strains isolated from carob tree-plantation soil. This fact can be the result of microorganism adaptation for carob beans.

Further study of initial screening strains; choice of *Aspergillus carbonarius* (AsDT10) for protein production :

Among the initial screening strains only those with tannin reduction between 42-70%, biomass yield (y = 45 %) and protein yield (yp = 18 %) were further cultured on medium B under the best possible culture conditions. These were *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Paecilomyces* strains.

The optimization of culture conditions improved the results for the investigated parameters by 28-142 % depending on the nature of the parameter as well as on the microorganism.

The microorganism eventually selected was *Aspergillus carbonarius* (AsDT10). This strain was isolated from decaying carobs and further cultured for the kinetics of growth and the chemical composition of the mycelium grown in medium C.

a. **Kinetics growth :**

The phase of vigorous growth was exponential (Fig. 1). The maximum value of specific growth rate $\mu^{max} = 0.300\ h^{-1}$ to a doubling time of 2.3 h was obtained in this phase. After 36 h of incubation the mycelium dry weight (24.5 mg/ml of medium) reached 75 % of the maximum which was observed at 72 h after inoculation. Biomass yield (y = 69.5 %), protein yield (yp = 25.6 %), dry weight biomass and specific growth rate observed in *A. carbonarius* cultures were significantly higher than the ones reported by SEKERI-PATARYAS & al. (1973), DROULISCOS & al. (1976) and MACRIS & KOKKE (1977) while y value is in agreement with that reported by MACRIS & KOKKE (1978).

The higher (40-50 %) value of y compared to values reported for synthetic media must be due to the fact that carob extract is a complex natural medium which in addition to sugars (used to calculate y) contains non carbohydrate compounds (e. g. tannins) which were consumed by the microorganism.

The initial concentration of total tannins in medium C (about 1/10 of total sugars) was by no means an inhibiting factor. On the contrary it improved growth of *A. carbonarius* while reduced tannins by 90 % after 60 h of incubation (Fig. 1). In *F. moniliforme* cultures reported by MACRIS & KOKKE (1977) the initial tannic acid concentration (about 18 mg per g carob sugar) had no effect on the mycelial growth and remained constant throughout the

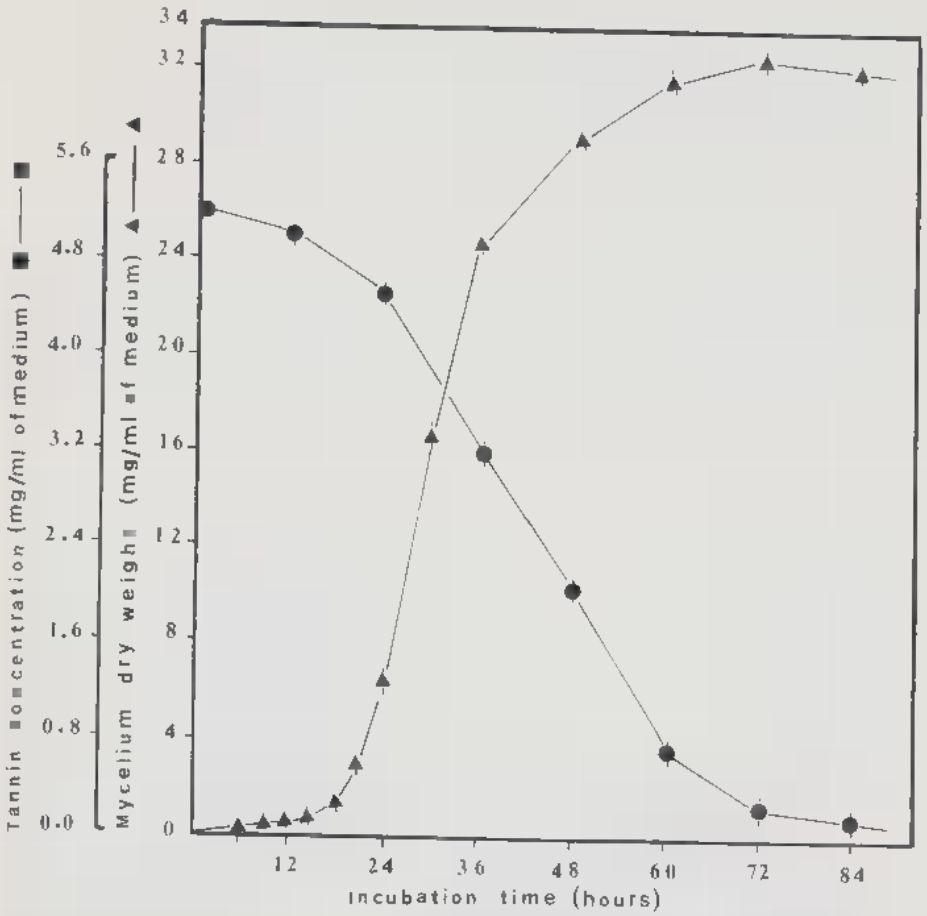

Fig. 1. — Changes in total tannin concentration and mycelium dry weight of *A. carbonarius* cultures in medium C in relation to incubation time.

Fig. 1. — Changements dans la concentration des tanins totaux et le poids sec du mycélium de cultures d'*A. carbonarius* dans le milieu C, en fonction du temps d'incubation.

incubation. According to this *A. carbonarius* proved to be significantly superior than *F. moniliforme* from the point of the utilization of tannins which constitute one of the major factors responsible for the elimination of carob nutritional value.

b. Gross composition of *A. carbonarius* biomass :

Gross composition of the dry mycelium is given in Table II. The true protein N comprises 80 % of the total mycelial N. This amount is at a level comparable to that reported by DROULISCOS & al. (1976) and SMITH & al. (1975). The

Components		%
Total N (TN)	:	7.2
Cold trichloracetic acid (TCA) soluble N	:	1.26
Alcohol insoluble ■	:	5.8
Non-protein N (NPN)	:	1.15
Crude protein (TN x 6.25)	:	45
True protein ((TN)-(NPN)) x 6.25	:	37.8
Lowry protein	:	37.3
DNA	:	0.4
RNA	:	4.7
Total nucleic acids	:	5.1
Total lipid	:	6.7
Ash	:	4.8
Total tannins	:	0.15
Moisture	:	4.9

Table II. – Gross composition (%) of *A. carbonarius* (AsDT10) biomass grown on medium C for 60 h.

Tableau II. – Composition (%) du mycélium sec de *A. carbonarius* cultivé pendant 60 h dans le milieu C.

mycelium true protein content is in agreement with that reported by MACRIS & KOKKE (1977) but significantly higher than the observed by SEKERI-PATARYAS & al. (1973), DROULISCOS & al. (1976). The cold TCA-soluble N fraction which contained mainly free amino acids and peptones constituted 18 % of the total N. The mycelium crude protein and ash content are considered to be acceptable. From the nutritional point of view these factors are important. The ash levels must be low, normally less than 5 %, in a compounded feed. The percentage of the biomass total lipids was about the same as observed in *F. moniliforme* biomass (MACRIS & KOKKE, 1978). The constituent fatty acids of the dry mycelium were : lauric, myristic, palmitic, palmitoleic, stearic, oleic and linoleic acid. Oleic and linoleic acids were abundant with proportion 60 % and 18 % respectively of the total. Thus mycelium lipids are rich in unsaturated fatty acids. The maximum RNA content of biomass was observed at mid-log phase while the minimum at stationary phase. The minimum RNA level prooved very low compared to published data HEDENSKOG & MOGREN (1973) and TREVELYAN (1975). This is a remarkable feature of this fungus because after 60 h of incubation and while RNA and attached tannin (carob originating) contents were minimum the total amount of mycelial protein (mg/ml) was maximum. Purine content (79.3 µmol/g of dry biomass) was lower than the

maximum observed in *F. moniliforme* mycelium (MACRIS & KOKKE, 1977) as well as that reported by TREVELYAN (1975) for baker's, commercial food yeast and *Rhizopus oryzae*. The percentage of the total mycelium attached tannins was 0.15 % of dry biomass. These tannins positively reacted with vanilin a fact which indicated their flavonol (catechin etc.) contents. Mycelium tannin content, practically non existed if culture time was increased from 60 to 84 hours.

The metabolizable energy of dry mycelium as well as its B-group vitamin content (Table III) satisfy the requirements for the production of microbial protein (SCP). Mycelium riboflavin content was double the one reported by FORAGE (1978). The metabolizable energy (18 MJ/kg of dry mycelium) was 69 % higher than the one reported by FORAGE (1978). This energy is sufficient for the daily requirement of growing rats (MALEFAKI-PERELA, 1981).

B_1	B_2	B_{12}	Biotin	Pantothenic acid	Nicotinic acid	Folic acid	Metabolizable energy
26	85	31	1.8	47	111	37	18

Table III. — B-group vitamin content (μg/g of biomass) and metabolizable energy (MJ/Kg of dry mycelium) of the *A. carbonarius* cultured on medium C for 60 h. (239 Kcal = 1MJ of metabolizable energy).

Tableau III. — Vitamines du groupe B (μg/g de biomasse) et énergie métabolisable (MJ/Kg de mycélium sec) de *A. carbonarius* cultivé dans le milieu C, pendant 60 h (239 Kcal = 1MJ de l'énergie métabolisable).

Amino acid composition of *A. carbonarius* biomass appears in Table IV. The amino acid profile indicated that the sulfur-containing amino acids were deficient compared to the requierments of the growing rat and the United Nations FAO/WHO (1965) reference protein. However compared to methionine and cystine content of other fungi (see Table IV) was higher. An examination of this Table reveals that most amino acids of *A. carbonarius* protein were predominant with the exception of tyrosine, serine and proline. It also becomes clear that valine, arginine, histidine and glutamic acid were significantly higher than those of *F. moniliforme*, *A. niger* and *A. oryzae*. The value of fluorodinitro benzene reactive lysine was 3.9 g/16gN which represented 74 % of the requirement of the growing rat.

Total essential amino acid content and essential amino acid index for *A. carbonarius* protein were higher than the ones of other fungi (Table IV), yeasts (DELANEY & al., 1975) and certain agricultural by-products vegetable origin (MALEFAKI-PERELA, 1981). Thus it becomes comparable or superior to many other suggested sources of fungal protein.

Amino acid	*A. carbonarius* (AsDT10)	*Aspergillus niger*[a]	*Fusarium moniliforme*[a]	*Aspergillus oryzae*[b]	Requirement of the growing rat[b]
	Grown on carob extract				
Phe	5.7	9.4	3.2	3.8	6.9
Tyr	4.3	5.6	7.3	5.0	
His	3.5	1.9	1.5	1.9	3.5
Ile	6.6	3.4	3.3	3.5	5.3
Leu	5.8	5.6	5.4	5.8	6.4
Lys	6.8	4.7	8.1	4.2	5.3
Met	1.9	1.6	0.9	1.3	4.2
Cys	1.5	0.5	-	1.0	
Thr	4.5	3.8	4.3	3.5	4.3
Val	8.4	4.8	4.2	4.6	5.3
Arg	9.5	5.6	4.9	4.4	1.8
Trp	0.7	0.9	-	1.4	1.0
Total essential amino acids	59.2	47.8	44.7	40.4	
Essential amino acid index	83.2	66.8	60.6	64.9	
Asp	8.5	6.8	7.0	6.9	
Ser	2.5	3.7	3.9	3.6	
Glu	14.5	9.5	10.8	12.4	
Pro	2.4	3.7	3.5	5.2	
Gly	4.1	3.9	4.1	3.7	
Ala	5.1	4.9	6.2	4.6	
Total amino acids	96.3	80.3	80.2	76.8	

a. DROULISCOS & al. (1976)

b. SMITH & al. (1975)

Table IV. – Amino acid composition (g/16 gN) of *A. carbonarius* biomass grown on medium C. Some other filamentous fungi are shown as well.

Tableau IV. – Composition en acides aminés (g/16 gN) de biomasse d'*A. carbonarius* cultivé dans le milieu C, ainsi que la composition d'autres champignons filamenteux.

Nutritional quality of *A. carbonarius* (AsDT10) biomass

The nutritional value of the *A. carbonarius* biomass was determined by feeding trials with rats using well established methods. Nutritional indices were : protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and true digestibility (TD). Results are given in Table V. PER is comparable to that reported for *F. moniliforme* (DROULISCOS & al., 1976). All indices (except PER) were comparable to those of soya bean oil meal (see Table V) and other protein sources of microbial or agricultural origin (DELANEY &

al., 1975; MALEFAKI-PERELA, 1981). NPU and BV were higher than the ones reported by SMITH & al. (1975) for several filamentous fungi. During the experimental period rats didn't lose their appetite.

Indices	Diets	
	A. carbonarius biomass	Soya bean oil meal
Protein efficiency ratio (PER)	1.95±0.1	2.6±0.15
Net protein utilization (NPU)	0.63±0.02	0.60±0.01
Biological value (BV)	0.70 ± 0.01	0.68 ± 0.02
True digestibility (TD)	0.85 ± 0.02	0.89 ± 0.01

Table V. — Nutritional indices of *A. carbonarius* biomass grown on medium C. The results were represented as mean values ± standard error.

Tableau V. — Indices de nutrition de *A. carbonarius* cultivé dans le milieu C. Les résultats représentent les valeurs moyennes ± erreur standard.

CONCLUSIONS

1. Deseeded carob pod extraction by autoclaving removed 75 % of the carob pod components more of which were sugars and tannins.
2. Microorganisms isolated from carobs and carob storehouse soil indicated high utilization of tannins.
3. *A. carbonarius* (AsDT10) strain appears potentially useful for fungal protein production because :
   - Grows fast and utilizes almost all sugars and total tannins in carob extract medium within 72 h of incubation.
   - Indicates high biomass (y) and protein yield (yp).

- Gross composition of biomass and amino acid profile are comparable or superior to those of the microorganisms which have been suggested for microbial protein production.
- The total mycelium protein and the amount of tannin used were significantly higher than those of other microorganisms studied by other scientists on the carob extract.
- The considerably low mycelium tannin content does not appear to present a toxicological problem or depress protein digestibility.

Finally, from the above, the following general remarks can be summarized :

a) The use of filamentous fungi was generally prefered because the recovery procedure of biomass after fermentation by filtration is more simple and cheap than the recovery of yeasts which has to be carried out by expensive centrifugation. Also, filamentous nature of the fungal biomass makes it suitable for human food.

b) The carob bean water soluble sugar and tannin extraction procedure is worthy even if we consider the extra cost for electricity, since the quantities extracted were much greater than those of other methods. In addition the spent carob (25 % of the carob pod weight) causes less environmental pollution.

c) If further nutritional tests with *A. carbonarius* mycelium, in accordance with the United Nations guidelines (1974), prove its safety, this microorganism could be used for microbial protein production from a low commercial and nutritional value agricultural product on the purpose of animal feeding. Such feeding trials are currently experienced on quail and rainbow trout.

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